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NOTES

Hexokinase Regulates Kinetics of Glucose Transport and Expression of Genes Encoding Hexose Transporters in *Saccharomyces cerevisiae*

THOMAS PETIT,[1,2] JASPER A. DIDERICH,[3] ARTHUR L. KRUCKEBERG,[3,4] CARLOS GANCEDO,[1] AND KAREL VAN DAM[3]*

Instituto de Investigaciones Biomédicas Alberto Sols, C.S.I.C.-UAM, 28029 Madrid, Spain,[1] and DSM Bakery Ingredients Division, 2600 MA Delft,[2] Swammerdam Institute for Life Science, 1018 TV Amsterdam,[3] and Department of Molecular Cell Physiology, Free University, 1081 HV Amsterdam,[4] The Netherlands

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Glucose transport kinetics and mRNA levels of different glucose transporters were determined in *Saccharomyces cerevisiae* strains expressing different sugar kinases. During exponential growth on glucose, a *hxk2* null strain exhibited high-affinity hexose transport associated with an elevated transcription of the genes *HXT2* and *HXT7*, encoding high-affinity transporters, and a diminished expression of the *HXT1* and *HXT3* genes, encoding low-affinity transporters. Deletion of *HXT7* revealed that the high-affinity component is mostly due to *HXT7*; however, a previously unidentified very-high-affinity component (K_m = 0.19 mM) appeared to be due to other factors. Expression of genes encoding hexokinases from *Schizosaccharomyces pombe* or *Yarrowia lipolytica* in a *hxk1 hxk2 glk1* strain prevented derepression of the high-affinity transport system at high concentrations of glucose.

The yeast *Saccharomyces cerevisiae* utilizes a variety of carbon sources for growth, but glucose and related hexoses are used preferentially. Glucose elicits a variety of responses that ensure its preferential use, from modulation of enzyme activity to repression or induction of genes (for reviews, see references 4 and 9). A great number of proteins participate in the process (for a review, see reference 9) of glucose repression, including hexokinase II, which is encoded by the gene *HXK2* (6, 7).

One of the activities regulated by glucose is sugar uptake. Glucose transport in *S. cerevisiae* is mediated by proteins encoded by several *HXT* genes (20; for reviews, see references 3 and 11). Glucose transport in yeast exhibits dual kinetics, with a high- and a low-affinity kinetic component (2) whose proportions depend on the culture conditions (5, 25). The kinetics observed are the result of the differential expression of the *HXT* genes, whose products have different affinities for glucose. *HXT1* and *HXT3* encode low-affinity transporters (K_m = 50 to 100 mM), *HXT2* and *HXT4* encode intermediate-affinity transporters ($K_m \sim$ 10 mM), and *HXT6* and *HXT7* encode high-affinity transporters (K_m = 1 to 2 mM) (19). Glucose represses *HXT* genes encoding high- and intermediate-affinity transporters and induces *HXT3* expression; these effects are relieved in *hxk2* mutants (12, 14, 18, 26). In this study, we have analyzed in parallel the kinetics of hexose uptake and the transcription of hexose transporter genes in *S. cerevisiae* strains carrying deletions in the *HXK2* gene and in strains expressing only *HXK2* or genes encoding the hexokinases from *Schizosaccharomyces pombe* or *Yarrowia lipolytica*.

We deleted the *HXK2* gene in the *S. cerevisiae* strain CEN.PK113-7D (*MAT***a** *MAL2-8*^{c} *SUC2*) to create strain KY116 and in strain CEN.PK113-5D (*MAT***a** *MAL2-8*^{c} *SUC2 ura3-52*) to create strain KY114, using the method of Wach et al. (24) and the primers AK53 (GTTGTAGGAATATAATTCTCCACACATAATAAGTACGCTAATTCGTACGCTGCAGGTCGAC) and AK54 (AAAAGGGCACCTTCTTGTTGTTCAAACTTAATTTACAAATTAAGTATCGATGAATTCGAGCTCG) (underlined nucleotides correspond to the DNA immediately 5′ and 3′ of the *HXK2* open reading frame, respectively). The *HXT7* gene was replaced in KY114 by *URA3* to produce strain KY168 via amplification of the *URA3* gene in plasmid pRS406 (22), using the primers JD3 (TATGCCAATACTTCACAATGTTCGAATCTATTCTTCATTTGCAGCGTATCACGAGGCCCTTTCGTC) and JD4 (ATGCACAAATTAGAGCGTGATCATGAATTAATAAAAGTGTTCGCAAAACGTTTACAATTTCCTGATGCGG) (underlined nucleotides correspond to DNA 5′ and 3′ of the *HXT7* open reading frame, respectively). In both cases correct disruption was checked by using an analytical PCR. To construct strains expressing only one hexokinase, the following plasmids were introduced into strain THG1 (*MAT***a** *leu2-1 ura3-52 hxk1*::*LEU2 hxk2*::*LEU2 glk1*::*LEU2*) (15): pCEN/*ScHXK2*, carrying the *S. cerevisiae HXK2* gene (17); pTP5, carrying the *S. pombe Sphxk2*$^+$ gene (encoding hexokinase 2) (15); or pDB20/*YlHXK1*, carrying the *Y. lipolytica YlHXK1* gene (16). The heterologous genes were under the control of the *S. cerevisiae ADH1* promoter. Hexokinase activity was measured as described previously (10). Cells were grown in batch at 250 rpm and 30°C in a minimal medium containing 2% (wt/vol) glucose, 0.1 M potassium phthalate (pH 5.0), and amino acids (21) as required. For transport assays, cells were harvested by centrifugation at 4°C (5 min, 4,000 × *g*), washed twice in ice-cold 0.1 M potassium phosphate buffer (pH 6.5), resuspended in this buffer to

* Corresponding author. Mailing address: Swammerdam Institute for Life Science, Plantage Muidergracht 12, 1018 TV Amsterdam, The Netherlands. Phone: 31(20) 525 5510. Fax: 31(20) 525 5505. E-mail: k.van.dam@chem.uva.nl.

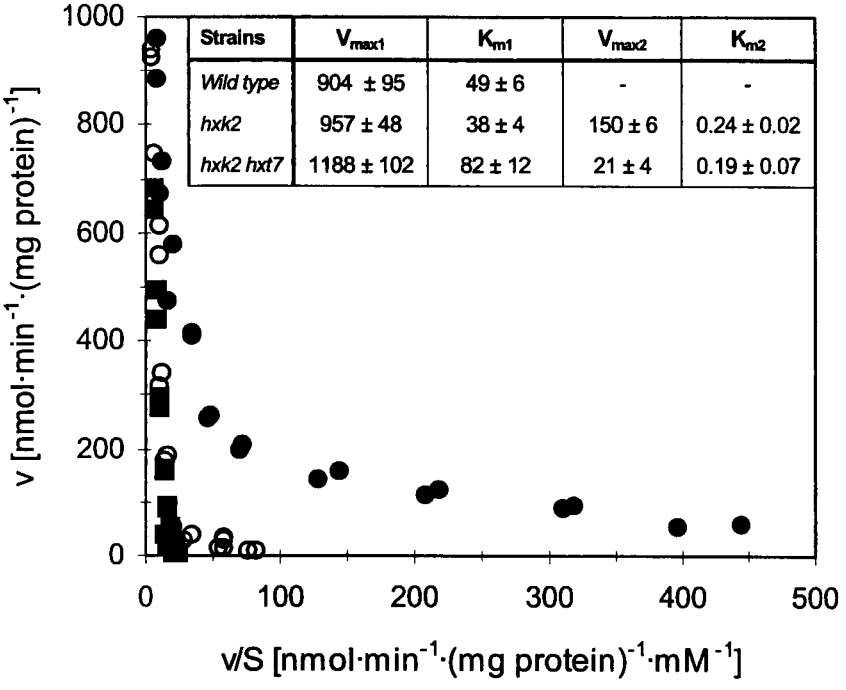

FIG. 1. Kinetics of glucose uptake in *S. cerevisiae* strains with successive deletions of the *HXK2* and *HXK7* genes. Zero *trans*-influx was determined for strain CEN.PK113-7D (wild type, ■), KY116 (*hxk2*Δ, ●), and KY168 (*hxk2*Δ *hxk7*Δ, ○) in cells from exponential cultures, as described in the text. S, extracellular glucose concentration; v, zero *trans*-influx rate of glucose. The kinetic parameters (shown in the inset) were calculated using the Enzfitter software. V_{max} is expressed in nanomoles per minute per milligram of protein, and K_m is in millimolar units.

a cell concentration of approximately 7.5 g of protein liter^{-1}, and kept on ice until use. The zero *trans*-influx rate of hexoses was determined according to the method described by Walsh et al. (25), at 30°C in 0.1 M potassium phosphate buffer (pH 6.5). Kinetic parameters of glucose transport were derived from least-squares fitting of the data to one- or two-component Michaelis-Menten models using Enzfitter software.

The kinetics of glucose uptake by *S. cerevisiae* cells depends on the stage of the culture; a low-affinity component is observed during exponential growth at high glucose concentrations, and a high-affinity component is observed when glucose is exhausted (1, 5, 25). During exponential growth in glucose, a wild-type strain and an isogenic *hxk2* mutant displayed a major low-affinity component with similar V_{max} and K_m values (Fig. 1). However, the *hxk2* strain showed, in addition, a very-high-affinity component with a K_m of 0.24 mM and a V_{max} that was about 16% of that of the low-affinity component (Fig. 1). No high-affinity component could be found in the wild type when the data were fitted to a two-component system. Fructose and mannose transport also showed an additional high-affinity component in cells of the *hxk2* mutant harvested during exponential growth on glucose (results not shown). Similar kinetics of glucose transport were obtained with strains of a different genetic background: DFY1 (*MAT***a** *lys1-1 leu2-1*) and the isogenic *hxk2* deletant DFY567 (*MAT***a** *lys1-1 leu2-1 hxk2*::*LEU2*).

When glucose was depleted, wild-type cells, as well as those of the *hxk2* mutant, displayed only high-affinity glucose uptake (K_m, around 2 mM). Also, no differences in the kinetics of fructose and mannose transport were found between the wild-type and *hxk2* mutant strains under these conditions (K_m for fructose, ca. 7 mM; K_m for mannose, ca. 14 mM). Deletion of the *HXT7* gene in the *hxk2* mutant eliminated a substantial proportion of the high-affinity component of glucose uptake during exponential growth on glucose (Fig. 1). However, in the double mutant, a component with very high affinity for glucose remained (K_m = 0.19 mM) but had a low activity (ca. 2% of that of the low-affinity component). This activity might be due to *HXT8* to *HXT17*. However, we favor the idea that the very-high-affinity component is due to the high level of *HXT2* expression observed in the *hxk2* strain grown at high glucose concentrations. This possibility is consistent with previous observations that suggested that the kinetics of Hxt2 for glucose is modulated by the growth conditions; it exhibits intermediate affinity in cells grown at high glucose concentrations while it presents dual kinetics, with a high- and a low-affinity component, in cells grown at low glucose concentrations (19).

We determined the abundance of *HXT* transcripts at different stages of growth on glucose by blotting hybridization with oligonucleotides highly specific for each *HXT* gene, as described previously (5) (Fig. 2). The wild-type strain expressed predominantly *HXT1* and *HXT3* during exponential growth on glucose, a result consistent with the low-affinity glucose uptake displayed (Fig. 1) (19). At the diauxic shift, transcription of the high-affinity transporter gene *HXT7*, and to a lesser extent *HXT6*, was increased and almost no mRNA corresponding to the low-affinity transporter genes *HXT1* and *HXT3* was detected. Under these conditions *HXT2* and *HXT4* mRNAs were not detected and *HXT5* mRNA was transcribed at a moderate level. In the *hxk2* deletion strain, high levels of *HXT2* and *HXT7* mRNA and reduced levels of *HXT1* and *HXT3* mRNA were observed during exponential growth on glucose; no mRNA corresponding to *HXT6* was detected. After glucose exhaustion, the levels of all *HXT* mRNAs, except that of *HXT5*, were quite low in the *hxk2* mutant. A similar expression pattern was observed using the DFY1 and DFY567 strains (results not shown).

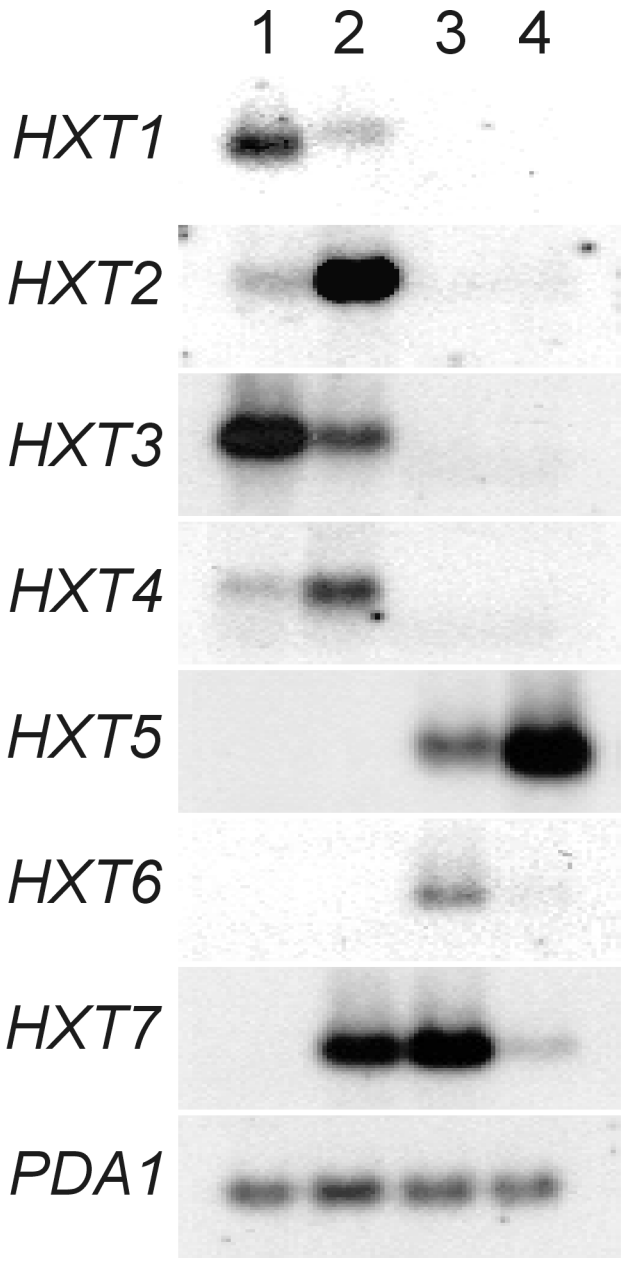

FIG. 2. Expression of different *HXT* mRNAs in *S. cerevisiae* wild-type (CEN.PK113-7D) and *hxk2*Δ (KY116) strains at different stages of growth on glucose. Cells were harvested simultaneously for RNA analysis and for the transport assays shown in Fig. 1. Lane 1, wild type, exponential growth (optical density at 600 nm = 1); lane 2, *hxk2*Δ, exponential growth (optical density at 600 nm = 1); lane 3, wild type, glucose exhaustion; lane 4, *hxk2*Δ, glucose exhaustion. The *PDA1* mRNA levels were used as controls for RNA loading (27).

The hexose uptake kinetics as well as the pattern of *HXT* transcription in glucose-grown cells shows that *HXK2* influences the expression of the *HXT* genes. During growth at high glucose concentrations, the deletion of *HXK2* strongly increases the high-affinity component of hexose transport and the expression of the *HXT2* and *HXT7* genes. This indicates that *HXK2* represses the appearance of the high-affinity component. Mutations in either *HXK1* or *GLK1* do not alter the expression pattern (13, 23). To determine the specificity of this effect of *HXK2*, we used *S. cerevisiae* strains that expressed only one gene encoding hexokinases from other yeast species.

An *S. cerevisiae* strain expressing only *S. cerevisiae HXK2* or the heterologous hexokinase genes from *S. pombe* or *Y. lipolytica* showed transport kinetics similar to that of a wild-type strain. During exponential growth on glucose, the strains displayed only low-affinity glucose transport. The best fit of the data was found by using a one-component system that yielded a mean (± standard deviation) V_{max} of 419 ± 30 and a K_m of 31 ± 3 for the strain expressing the *S. pombe hxk2*$^+$ gene and a V_{max} of 496 ± 44 and a K_m of 23 ± 3 for the strain expressing the *Y. lipolytica HXK1* gene (V_{max} and K_m are expressed in nanomoles per minute per milligram of protein and in millimolar units, respectively). These results indicate that the heterologous hexokinases can replace the *S. cerevisiae* protein in exerting glucose repression on high-affinity glucose uptake. The heterologous hexokinases are also active in invertase repression (16).

The presence in *S. cerevisiae* of a large family of glucose transporters that have different affinities for their substrates and whose expression is finely regulated remains an enigma. From a physiological point of view it appears reasonable that high-affinity transporters are expressed only at low external glucose concentrations. However, it is not immediately clear how expression of these transporters at high glucose concentrations could be detrimental to the cell.

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